TABLE 1. Sources of plants for cpDNA study of Solanaceae.

Species	Source[a]	Voucher information[b]
Solanaceae		
Cestroideae		
Anthocercideae		
1. *Anthocercis viscosa*	Symon	DES 14835
2. *Cyphanthera anthocercidea*	Symon	DES 14836
3. *Duboisia myoporoides*	Symon	DES 14832
4. *Grammosolen dixonii*	Symon	DES 14833
Cestreae		
5. *Cestrum nocturnum*	Matthaei	21314
6. *Vestia lyciodes*	Lester	BIRM/S.0105
Nicotianeae		
7. *Fabiana imbricata*	UCSB	81342
8. *Nicotiana tabacum*	Matthaei	no voucher
9. *Petunia axillaris*	Lester	BIRM/S.0367
Salpiglossideae		
10. *Browallia speciosa*	Lester	BIRM/S.0416
11. *Brunfelsia americana*	Matthaei	840215
12. *Salpiglossis sinuata*	Lester	BIRM/S.0181
13. *Schizanthus pinnatus*	Lester	BIRM/S.0224
14. *Streptosolen jamesii*	JBB	RGO S-106
Solanoideae		
Datureae		
15. *Brugmansia sanguinea*	JBB	RGO S-7
16. *Datura stramonium*	Olmstead	RGO S-16
Hyoscyameae		
17. *Hyoscyamus albus*	Lester	BIRM/S.1218
Juanulloeae		
18. *Dyssochroma viridiflora*	Brown	s.n.
19. *Hawkesiophyton panamensis*	Lester	BIRM/S.1462
20. *Juanulloa aurantiaca*	Lester	BIRM/S.0411
Lycieae		
21. *Grabowskia duplicata*	Lester	BIRM/S.0258
22. *Lycium cestroides*	Lester	BIRM/S.0368
Nicandreae		
23. *Nicandra physaloides*	BealBG	RGO S-38
Solandreae		
24. *Solandra grandiflora*	Matthaei	840415
Solaneae		
25. *Atropa belladona*	Lester	BIRM/S.0078
26. *Capsicum baccatum*	Eshbaugh	WHE 1584
27. *Chamaesaracha coronopus*	Turner	BLT 15854
28. *Cyphomandra betacea*	Bohs	Nee 30359
29. *Exodeconus miersii*	Lester	BIRM/S.1223
30. *Jaltomata edulis*	BealBG	RGO S-24
31. *Lycianthes lycioides*	JBB	RGO S-87
32. *Lycopersicon esculentum*	Palmer	no voucher
33. *Mandragora officinalis*	Lester	BIRM/S.0672
34. *Margaranthus solanaceous*	Lester	BIRM/S.0610
35. *Physalis alkekengi*	D'Arcy	WGD 17707
36. *Saracha spinosa*	UCB	75.0784
37. *Solanum carolinense*	Lester	BIRM/S.1816
38. *Solanum luteoalbum*	Bohs	BIRM/S.0042
39. *Solanum americanum*	Olmstead	RGO S-94
40. *Solanum candidum*	Lester	BIRM/S.0975
41. *Withania coagulans*	Lester	BIRM/S.0678

TABLE 1. Continued.

Species	Source[a]	Voucher information[b]
Nolanoideae		
42. *Nolana spathulata*	Dillon	MOD 3767
Convolvulaceae		
43. *Ipomoea coccinea*	BealBG	RGO s.n.

[a] BealBG = Beal Botanical Garden, Michigan State University, Bohs = Lyn Bohs, Brown = Keith S. Brown, D'Arcy = William G. D'Arcy, Dillon = Michael O. Dillon, Eshbaugh = W. Hardy Eshbaugh, JBB = Jardín Botánico de Bogotá, Lester = Richard N. Lester, Matthaei = Matthaei Botanic Garden, Olmstead = Richard G. Olmstead, Palmer = Jeffrey D. Palmer, Symon = David E. Symon, Turner = B. L. Turner, UCB = University of California, Berkeley, Botanical Garden, UCSB = University of California, Santa Barbara, greenhouse.

[b] Numbers preceded by initials or name indicate collector (BLT = Turner, DES = Symon, Hawkes = J. G. Hawkes, MOD = Dillon, Nee = Michael Nee, RGO = Olmstead, WGD = D'Arcy, WHE = Eshbaugh) and collection number. Material provided by Richard N. Lester bears the accession number of the University of Birmingham Solanaceae Collection. All other numbers are accession numbers for living collections at botanical gardens.

Solanaceae and the more distantly related outgroups precluded effective comparative mapping of large portions of their genomes.

RESULTS

A total of 1,074 different restriction sites was identified among the 43 taxa included. Of these, 194 sites (18.1%) were invariant, 433 (40.3%) were present or absent in all but one species, and therefore did not provide information concerning relationships among taxa, and 447 (41.6%) were phylogenetically informative (a complete data matrix is available upon request from R. Olmstead). The distribution of informative restriction sites was nonrandom. The inverted repeat portion of the genome, which accounts for approximately 20% of the genome complexity, accounts for 44% of the invariant sites and only 6% of the phylogenetically informative sites. This extreme conservation of restriction sites in the inverted repeat is in accord with results from studies of nucleotide sequence evolution (Wolfe et al., 1987) and suggests that comparative restriction-site mapping of the inverted repeat can be applied to problems at greater phylogenetic depth than the genome as a whole (Downie & Palmer, 1992).

Fourteen restriction-fragment length variants, representing probable insertions and deletions ranging in size from 150 to 700 base pairs, were mapped. Five of these length variants are shared by two or more taxa. The length variants were not used directly in the phylogenetic analysis, but extremely accurate mapping, made possible by reference to the completely sequenced tobacco genome, enabled the identification of restriction sites whose presence or absence was the product of the insertion or deletion, and those sites were scored as missing data for those taxa. The five shared length variants are all implied to be deletions by the results of the phylogenetic analysis (Fig. 2). Four of the five variants represent unique events; the fifth deletion is implied to have occurred in parallel on four separate occasions (Fig. 2). Numerous smaller insertions/deletions (<100 bp) were detected, but not mapped.

The phylogenetic analysis conducted using Wagner parsimony (all restriction site changes weighted equally) resulted in 45 equally parsimonious trees with a length of 1,227 and a consistency index of 0.36 excluding autapomorphies, and 0.53 including autapomorphies (Kluge & Farris, 1969), from which a strict consensus tree was constructed (Fig. 2). A bootstrap analysis (Felsenstein, 1985) was conducted with 100 replications to provide a measure of support for the monophyletic groups identified in the consensus tree (Fig. 2). The bootstrap majority rule consensus tree was entirely congruent with the consensus tree from the Wagner parsimony analysis.

Figure 3 shows one of the 45 most parsimonious trees to illustrate the distribution of character support (putative gains or losses of restriction sites) for clades on the tree. Both trees (Figs. 2, 3) show an ancestral, paraphyletic Cestroideae and a derived monophyletic Solanoideae. The earliest branch consists of the morphologically divergent genus *Schizanthus*, while the rest of the Cestroideae comprise three distinct lineages. The Nicotianeae are split among two of the distinct cestroid lineages, with *Nicotiana* forming a clade with the Australian endemic tribe Anthocercideae, whereas *Fabiana* and *Petunia*, along with *Brunfelsia*, constitute another clade. Members of the Salpiglossideae are found on three of the four lineages of Cestroideae

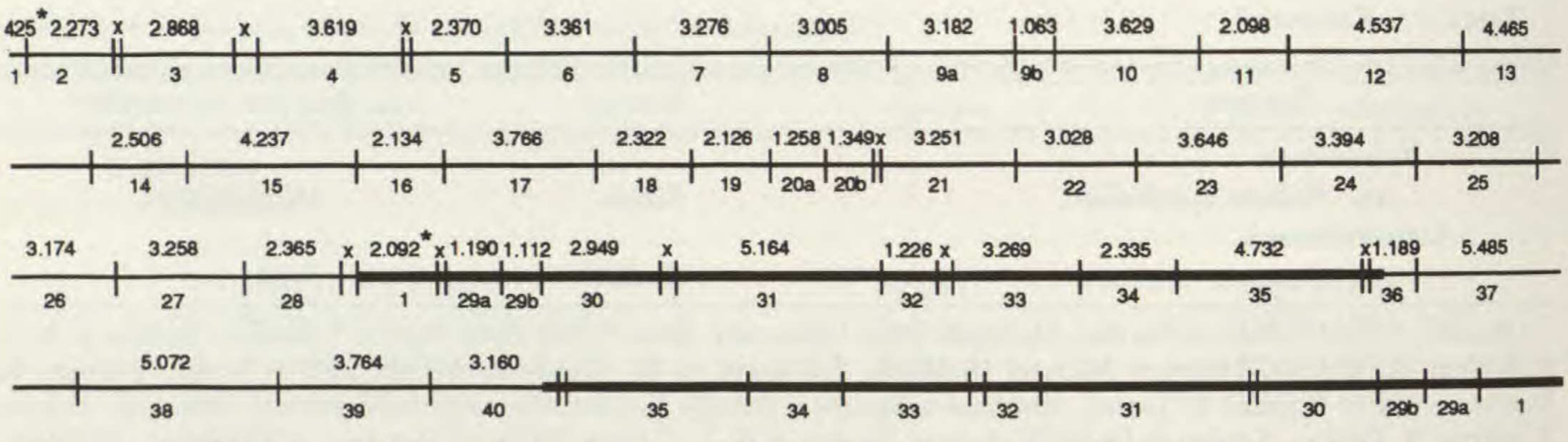

FIGURE 1. Linear map of *Nicotiana tabacum* cpDNA (modified from Shinozaki et al., 1986). The inverted repeat is indicated by the heavy line. SolClone Top40 clone bank fragments are indicated by number (Table 2) below the line and size in kb above the line. Fragments indicated by "x" are not included in the clone bank. SolClone #1 spans the junction between the large single copy region and inverted repeat; the portions belonging to each are indicated by an asterisk.

represented on both trees, supporting D'Arcy's (1978) suggestion that the tribe is artificial.

The major unresolved portion of the consensus tree falls exactly at the point of divergence of the Solanoideae. Several lineages within the Solanoideae correspond to currently recognized tribes, including the Lycieae, Datureae, and Juanulloeae. The large tribe Solaneae appears not to be a monophyletic group. Three genera placed in the Solaneae by D'Arcy (1979, 1991), *Mandragora*, *Atropa*, and *Exodeconus*, fall outside the clade containing most of the representatives of the tribe, whereas the Datureae fall within that clade. *Nolana* (Nolanaceae or Nolanoideae) branches within the Solanoideae, closest to the tribe Lycieae.

A further analysis was performed to test the strength of the result that the Nicotianeae are split between two distinct cestroid lineages by imposing the constraint that the Nicotianeae plus Anthocercideae form a monophyletic group. Including the Anthocercideae in the constraint provided a more liberal test than that of strict monophyly for the Nicotianeae, yet the shortest tree (not shown) was still 16 steps longer than the most parsimonious tree.

Relationships among a larger number of taxa in the Solanoideae are the subject of an analysis currently in progress, but two steps were taken in the current study to try to increase resolution within the Solanoideae. First, to reduce the amount of homoplasy that inevitably arises among distantly related taxa, the cestroid lineage closest to the Solanoideae (Fig. 2) was chosen as a functional outgroup (Watrous & Wheeler, 1981) for an analysis with a reduced number of taxa. The resulting consensus tree of three equal trees (Fig. 4) is congruent with the tree produced by the complete analysis (Fig. 2), but completely resolves the polychotomy at the base of the Solanoideae produced by the global analysis. This tree (Fig. 4) suggests a basal branch of the Solanoideae comprising *Exodeconus* and *Nicandra*. The rest of the taxa form two main clades. One clade consists of the Datureae and most of the Solaneae (as in the global analysis). The other clade consists of two groups, the Solandreae, Juanulloeae, and *Mandragora* in one and the Hyoscyameae, Lycieae, *Atropa*, and *Nolana* in the other.

The second approach to increasing resolution involved varying the assumptions concerning evolution of restriction sites, specifically that there exists a greater probability of loss than of gain of any one restriction site. Asymmetric weighting was implemented using the Stepmatrix option of PAUP with weights for gain : loss of 1.3 : 1.0 as recommended by Albert et al. (1991). The single shortest tree from this analysis (Fig. 5) is congruent with one of the three trees found using the functional outgroup approach, except that the clade comprising the Hyoscyameae, Lycieae, *Atropa*, and *Nolana* groups with the Solaneae in the weighted analysis rather than with the Solandreae, Juanulloeae, and *Mandragora*.

DISCUSSION

CESTROIDEAE

It is clear from the phylogenetic analysis (Fig. 2) that the Cestroideae are the ancestral subfamily of Solanaceae. Traditional views of the family have considered the Solanoideae as ancestral based on a priori assumptions about trends in the evolution of characters from "primitive" to "specialized" that have been postulated to exist throughout the angiosperms (Melchoir, 1964; D'Arcy, 1979). That reversals in these "trends" may characterize particular groups, as is apparent from this analysis in

TABLE 2. SolClone Top40 clone bank[a] constructed from *Nicotiana tabacum* cpDNA.

	Subclone[b]	Size (kb)	Coordinates[c]	Vector[d]
1.	Bam 8—BamHI-SpeI(XbaI)	2.517	153746–419	pTZ19R
2.	Bam 8—SpeI(XbaI)-BamHI	2.273	419–2692	pTZ19R
3.	Bam 15	2.868	2832–5700	pTZ19R
4.	Bam 10a	3.619	6149–9768	pTZ19R
5.	Bam 4—BamHI-SacI	2.370	9935–12305	pTZ19R
6.	Bam 4—SacI-SacI	3.361	12305–15666	BSsk+
7.	Bam 4—SacI-BamHI	3.276	15666–18942	pTZ19R
8.	Bam 13	3.005	18942–21947	pTZ19R
9a.	Bam 12a	3.182	21947–25128	BSsk+
9b.	Bam 25	1.063	25128–26191	BSsk+
10.	Bam 10b	3.629	26191–29820	pTZ19R
11.	Bam 19	2.098	29820–31918	BSsk+
12.	Bam 9a	4.537	31918–36455	pBR322
13.	Bam 9b	4.465	36455–40920	BSsk+
14.	Bam 16	2.506	40920–43426	pTZ19R
15.	Bam 3—BamHI-SacI	4.237	43426–47699	BSsk+
16.	Bam 3—SacI-SaII	2.134	47699–49833	BSsk+
17.	Bam 3—SaII-BamHI	3.766	49833–53599	BSsk+
18.	Bam 17	2.322	53599–55921	pTZ19R
19.	Bam 18	2.126	55921–58047	pTZ19R
20a.	Bam 22c	1.258	58047–59305	pTZ19R
20b.	Bam 20	1.349	59305–60654	pTZ19R
21.	Bam 12b	3.251	60850–64101	pTZ19R
22.	Bam 1—BamHI-SacI	3.028	64101–67129	pTZ19R
23.	Bam 1—SacI-SaII	3.646	67129–70773	pTZ19R
24.	Bam 1—SaII-EcoRV	3.394	70773–74167	BSsk+
25.	Bam 1—EcoRV-SacI	3.208	74167–77375	BSsk+
26.	Bam 1—SacI-BglII	3.174	77375–80549	BSsk+
27.	Bam 1—BglII-BamHI	3.258	80549–83807	BSsk+
28.	Bam 7—BamHI-PstI	2.365	83807–86172	pTZ19R
29a.	Bam 23b	1.190	88991–90181	pTZ19R
29b.	Bam 24b	1.112	90181–91293	pTZ19R
30.	Bam 14b	2.949	91293–94242	pTZ19R
31.	Bam 6b	5.164	94562–99726	pBR322
32.	Bam 22b	1.226	99726–100952	pTZ19R
33.	Bam 11b	3.269	101532–104801	pTZ19R
34.	Bam 5b—BamHI-NheI(XbaI)	2.335	104801–107136	BSsk+
35.	Bam 5b—NheI(XbaI)-BamHI	4.732	107136–111868	BSsk+
36.	Bam 21	1.189	111924–113113	pTZ19R
37.	Bam 2—BamHI-XhoI	5.485	113119–118604	BSsk+
38.	Bam 2—XhoI-PstI	5.072	118604–123676	BSsk+
39.	Bam 2—PstI-SacI	3.764	123676–127440	BSsk+
40.	Bam 2—SacI-BamHI	3.160	127440–130600	BSsk+

[a] These clones may be obtained by writing to J. Palmer.

[b] Subclones are derived from parent clones provided by M. Sugiura (Sugiura et al., 1986) and are either BamHI clones or subclones derived from BamHI clones as noted. CpDNA restriction sites not found in the multiple-cloning region of vectors pTZ19R and BSsk+ are cloned into sites noted in parentheses. Each set of paired subclones (9a+b, 20a+b, and 29a+b) is intended to be used as a single hybridization probe. The one large gap in coordinate coverage (between clones 28 and 29a) represents one end of the large inverted repeat and is covered by clone 1. Ten smaller gaps, nine of which are shown in Figure 1, totaling 2641 bp in size, are not covered by this clone bank.

[c] Coordinates for the *Nicotiana tabacum* cpDNA sequence are those of Shinozaki et al. (1986).

[d] All plasmids are ampicillin-resistant.

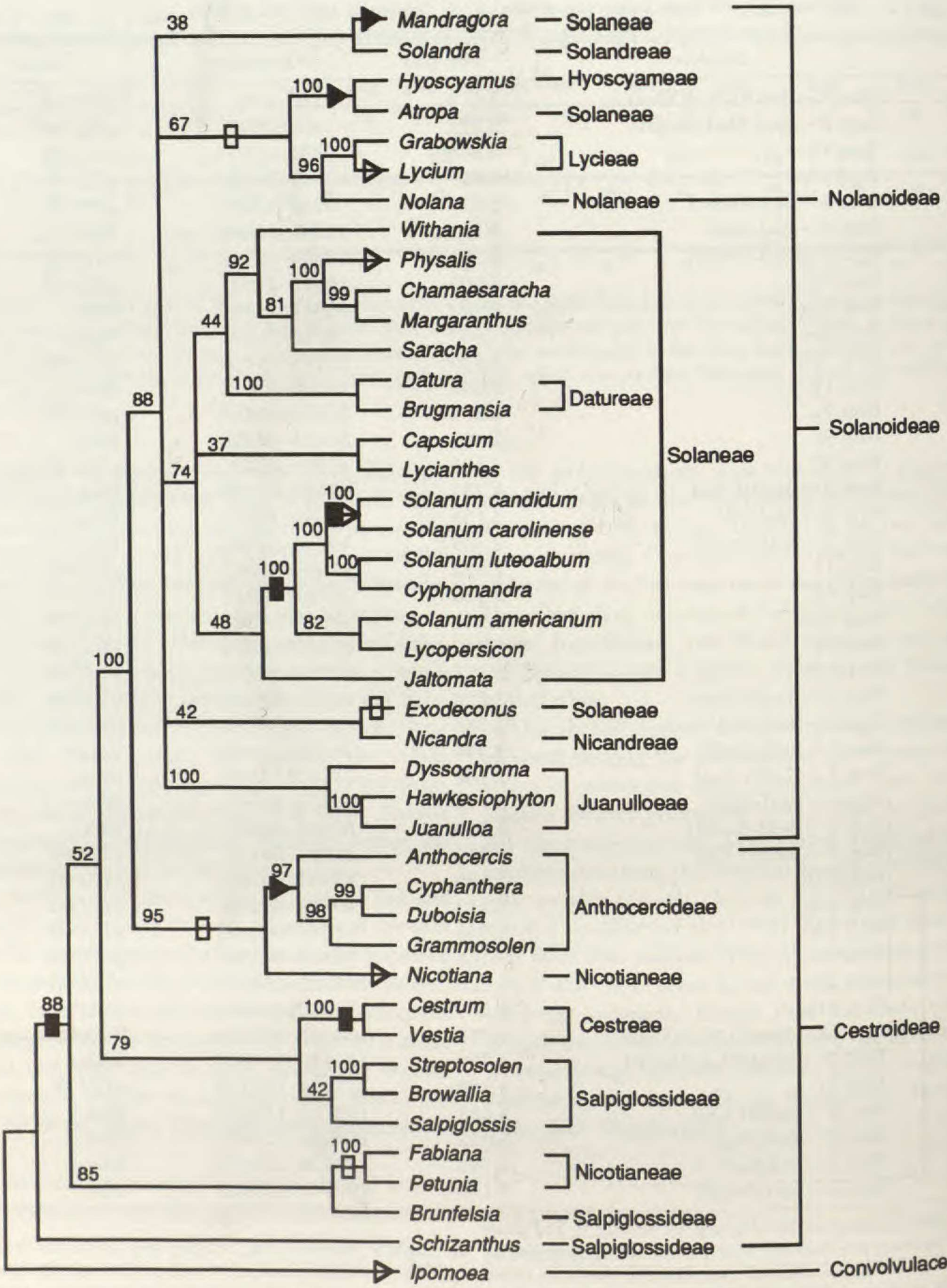

FIGURE 2. Strict consensus of 45 equally parsimonious trees derived from the Wagner parsimony analysis of 447 informative restriction sites in the Solanaceae. The percentage of bootstrap replicates supporting each clade is indicated along the internode for that clade. Tribal and subfamilial designations follow D'Arcy (1991). Solid triangles indicate groups with strictly Old World or Australian native distributions. Open triangles indicate groups with New World and Old World native distributions. All others are New World only. Note that not all Solanoideae taxa with New/Old World distributions are included in this analysis. Rectangles indicate mapped deletions, which were not used to infer phylogeny; solid rectangles indicate unique deletions, and open rectangles indicate homoplastic occurrences of one deletion (see text).

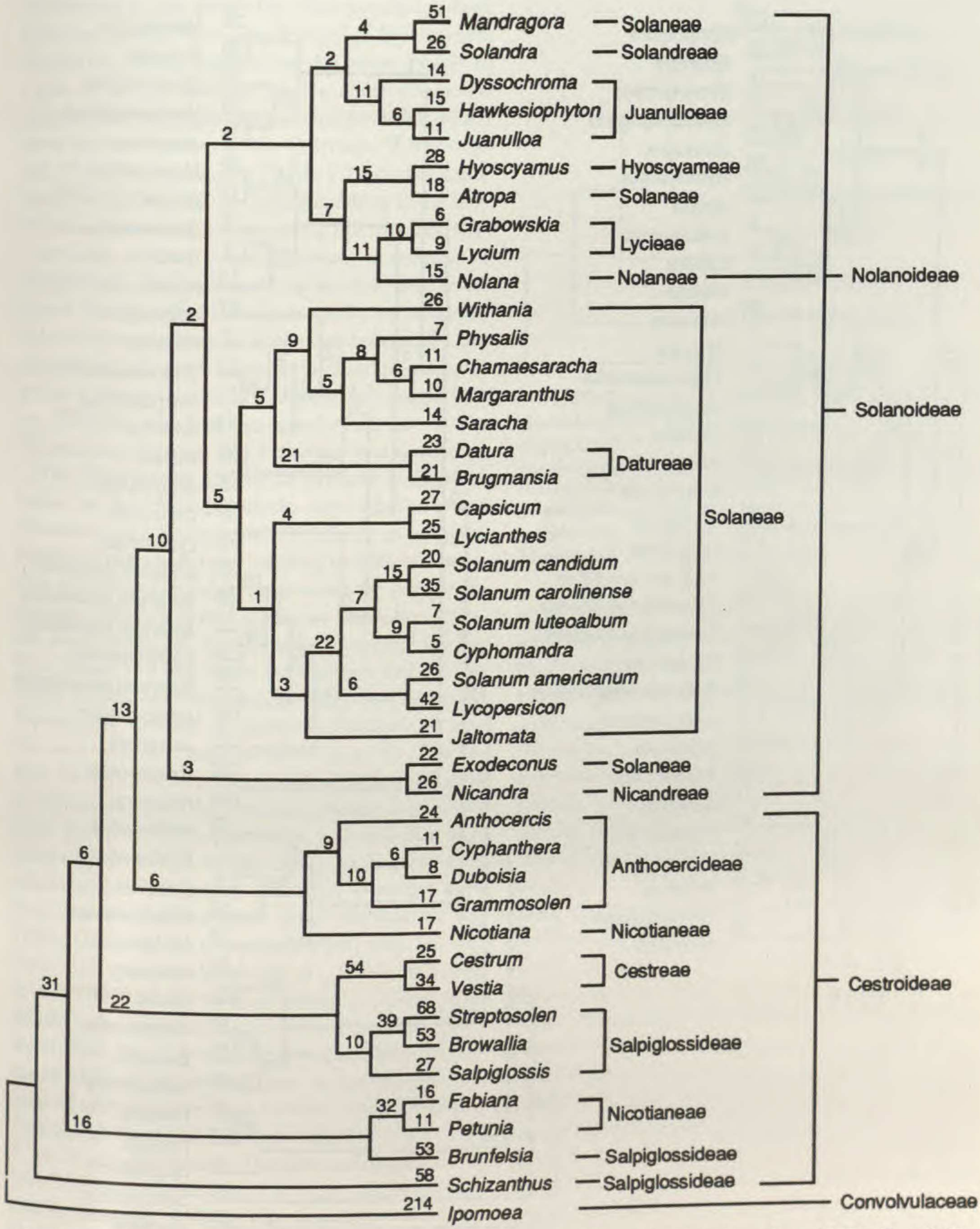

FIGURE 3. One of the 45 equally parsimonious Wagner trees of the Solanaceae (length = 1227, CI = 0.36, both calculated excluding autapomorphies). Total tree length, including 433 autapomorphies, is 1660. Number of restriction site changes supporting each clade is indicated. Terminal branch lengths include autapomorphies and implied homoplasies.

the Solanaceae, could not be tested without a rigorous phylogenetic analysis. Four of the five recognized tribes of Cestroideae are represented in the analysis. Material of the fifth tribe, Schwenckieae, was not available at the time of this study. The representatives of two of the tribes, Cestreae and Anthocercideae, correspond to clades on the tree, whereas the two other tribes, Salpiglossideae and Nicotianeae, do not. The Salpiglossideae, characterized by their zygomorphic floral symmetry, are

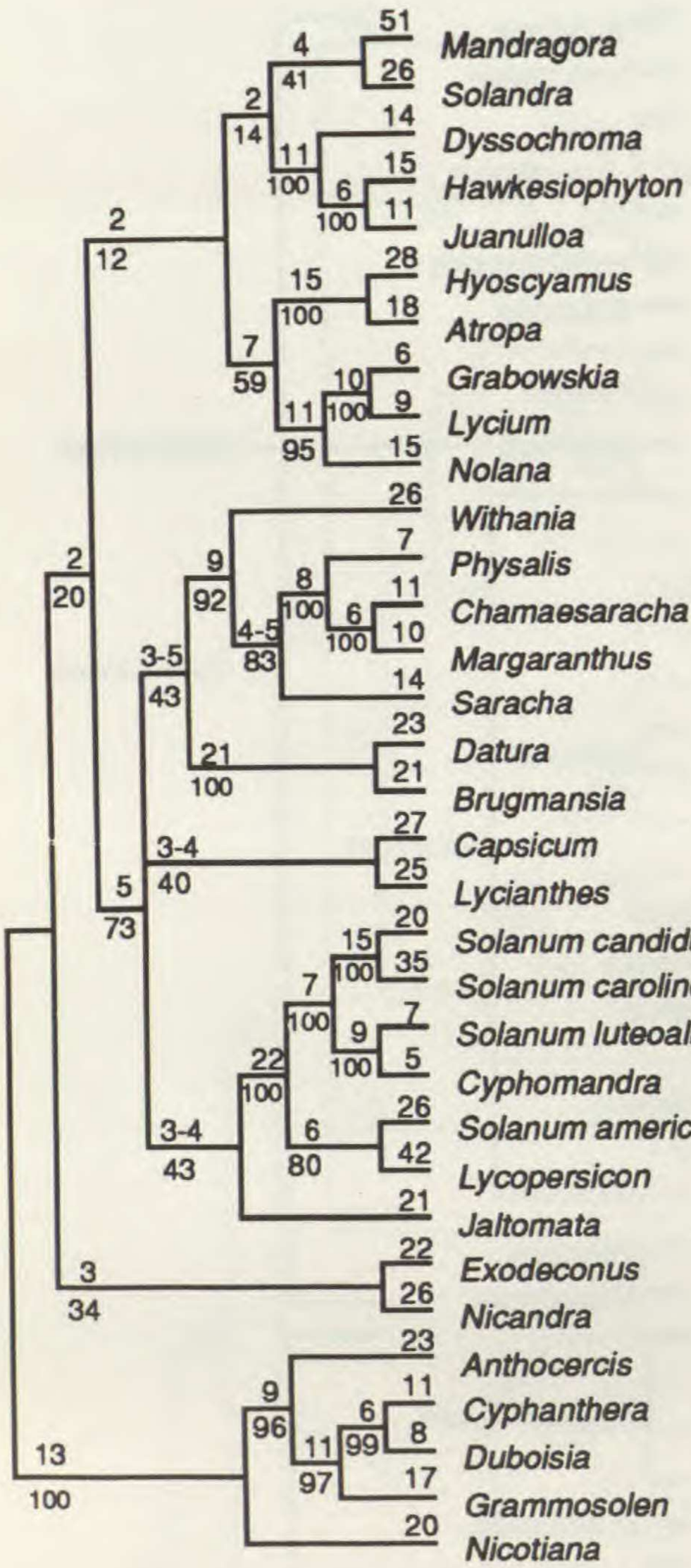

Fig. 4

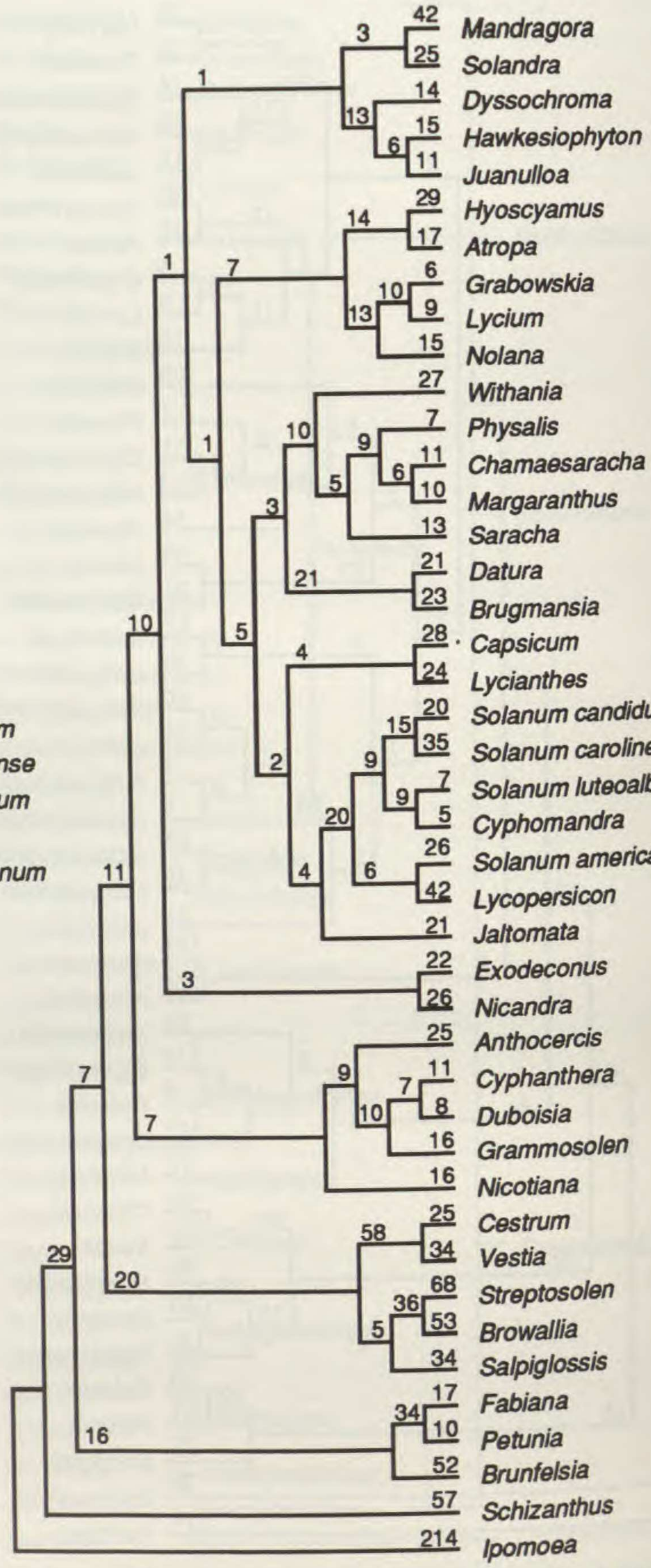

Fig. 5

FIGURES 4, 5.—4. Strict consensus of three equally parsimonious Wagner trees of the subfamily Solanoideae using its sister clade comprised of *Nicotiana* and the Anthocercideae as a functional outgroup (length = 629, CI = 0.441, excluding autapomorphies). Total tree length, including 249 autapomorphies, is 878. Number of restriction site changes supporting each clade is indicated. Terminal branch lengths include autapomorphies and implied homoplasies. The branch lengths for the three alternate topologies are indicated by a range of values. The percentage of bootstrap replicates supporting each clade is indicated beneath the internode for that clade.—5. The single most parsimonious tree resulting from asymmetric weighting of gains vs. losses of 1.3 : 1.0. This tree is one of the 45 equally parsimonious Wagner trees. Number of restriction site changes supporting each clade is indicated.

represented in this study by: *Schizanthus*, which forms the earliest diverging lineage of Solanaceae; *Brunfelsia*, which appears as the sister group to a part of the Nicotianeae; and a clade composed of *Browallia*, *Salpiglossis*, and *Streptosolen*, which forms the sister group to the Cestreae. We concur with D'Arcy's argument (1978, 1979) that taxonomic recognition of the Salpiglossideae in its current broad sense is unwarranted, but suggest that a narrowly defined Salpiglossideae, consisting of *Salpiglossis*, *Browallia*, *Streptosolen*, and other genera closely related to them might be retained. *Schizanthus* stands apart both morphologically and phylogenetically and appears to warrant recognition as a monogeneric tribe. *Brunfelsia* might best be combined with the excluded elements of the Nicotianeae, *Petunia* and *Fabiana*, in a new tribe.

The Nicotianeae appear to present a situation similar to the Salpiglossideae, with *Fabiana* and *Petunia* in one lineage and *Nicotiana* in another. However, the characters uniting the Nicotianeae, such as herbaceous habit, actinomorphic floral morphology, nonarticulated pedicels, capsular fruits, and small seeds, all appear to be ancestral for the Cestroideae and the entire Solanaceae and are retained in separate lineages, rather than being independently derived in different lineages as was the case with the Salpiglossideae (Fig. 6). The results presented here indicate that a clade composed of *Nicotiana* and the Anthocercideae forms the sister group to the Solanoideae, a conclusion supported by 100% of the bootstrap replicates. This relationship is congruent with DNA sequence data from the chloroplast *rbc*L gene (Palmer et al., 1988; Olmstead et al., unpublished) and the nuclear *rbc*S gene (Pichersky et al., 1986; Meagher et al., 1989), from both of which a closer relationship was inferred for *Nicotiana* and *Lycopersicon* than for either with *Petunia*. The Nicotianeae also can be divided into two groups on the basis of chromosome base number, with *Fabiana*, *Petunia*, *Latua*, and *Nierembergia* having $x = 7$, 8, or 9 and *Nicotiana*, *Combera*, *Pantacantha*, and *Benthamiella* having $x = 11$ or 12 (Hunziker, 1979; Moscone, 1989). The transition series for chromosome base number in the family is not clear as to the disposition of $x = 11$ (see discussion below). However, the evolution of base number $x = 12$ appears to provide a synapomorphy for the clade comprised of *Nicotiana*, the Anthocercideae, and the Solanoideae (Fig. 6).

The Anthocercideae and *Nicotiana* are sister groups on the cpDNA tree. A substantial amount of molecular data, including a large (ca. 650 bp) deletion in the cpDNA unique to *Nicotiana* (Olmstead et al., 1990), indicates that both *Nicotiana* and the Anthocercideae are monophyletic. The Australian species of *Nicotiana* form a derived clade within *Nicotiana* and are very homogeneous with respect to their cpDNA (Olmstead et al., 1990). *Nicotiana* is well represented in Australia, where the Anthocercideae are endemic, but there is much greater morphological and cpDNA divergence among the members of the Anthocercideae than among the Australian species of *Nicotiana* (Olmstead et al., 1990). This combination of phylogenetic inference, biogeographic distribution, and cpDNA divergence leads to the following conclusions: (1) the most recent common ancestor of the two groups was South American and (2) two separate colonizations of Australia occurred, one in the proto-Anthocercideae, prior to diversification of that lineage, and one late in the diversification of *Nicotiana*. The amino acid sequence data of Martin & Dowd (1984) also suggest a recent arrival of *Nicotiana* in Australia. These two introductions of Solanaceae into Australia resulted in the only cestroid elements native outside of the New World (the single species of *Nicotiana* in Africa is believed to be a secondary immigrant from Australia; Martin & Dowd, 1984).

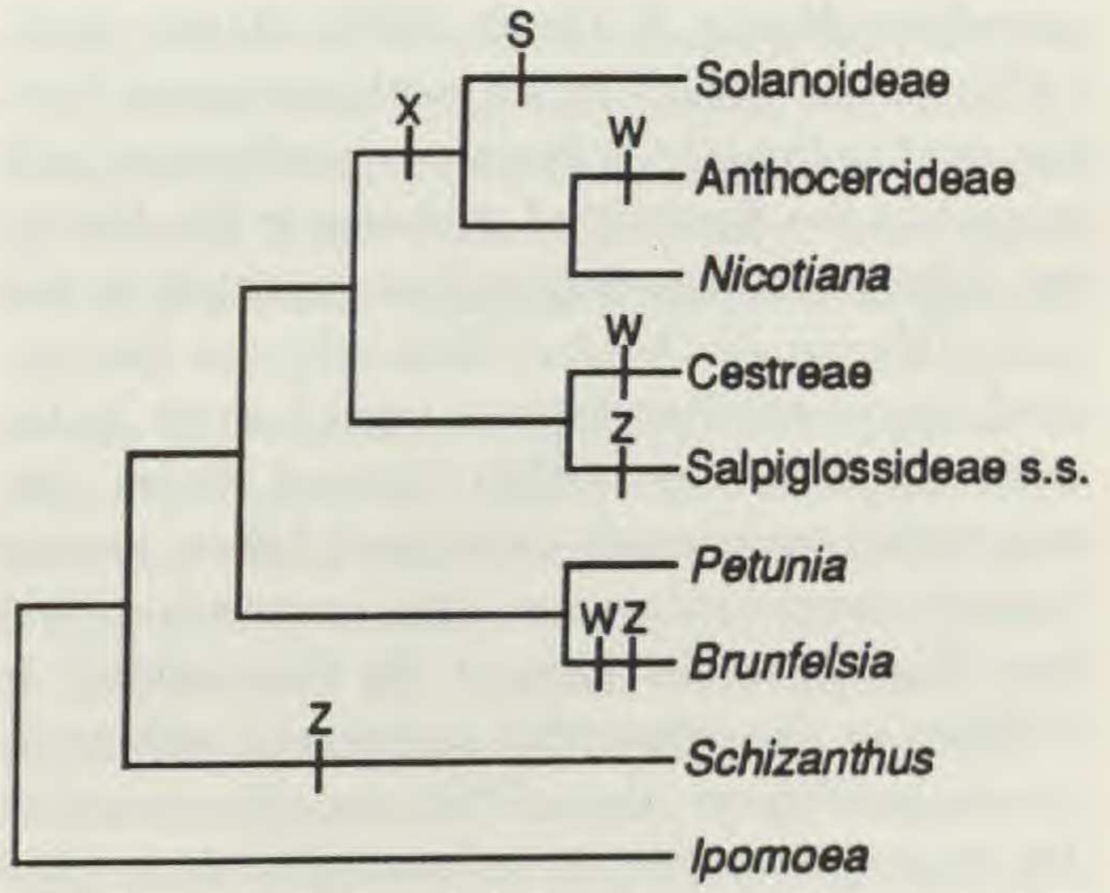

FIGURE 6. Reduced cladogram of the Solanaceae with character state transformations for some prominent morphological (S = seed discoidal with curved embryo, W = woody habit, Z = zygomorphic floral symmetry) and chromosomal (X = base chromosome number $x = 12$) characters indicated. Note the absence of morphological apomorphies on the lineages leading to members of the tribe Nicotianeae, *Nicotiana* and *Petunia*.

SOLANOIDEAE

The cpDNA tree indicates that the subfamily Solanoideae is monophyletic and derived from the Cestroideae, contrary to previously held views (D'Arcy, 1979, 1991). The results of Martin and

coworkers (Martin & Dowd, 1984; Martin et al., 1984), using partial amino acid sequences from the small subunit of rubisco, are ambiguous with respect to the direction of evolution in the family; the Solanoideae are depicted as ancestral in one study (Martin & Dowd, 1984), whereas the cestroid representatives form a basal branch in the other (Martin et al., 1984). Several clades, corresponding to currently recognized tribes, emerge from the unresolved portion of the consensus cpDNA tree (Fig. 2) at the base of the Solanoideae. In addition to the substantial agreement with traditional classification, the cpDNA tree offers solutions for some genera that have been difficult to place in the current classification. D'Arcy places *Atropa* and *Mandragora* in the Solaneae "more for convenience than conviction" (D'Arcy, 1991). The present study unambiguously places *Atropa* with *Hyoscyamus* of the Hyoscyameae, in agreement with Tetenyi (1987). *Mandragora* is isolated in the cpDNA analysis, at best only loosely related to *Solandra*, and is remote from either the Solaneae or the *Atropa*/Hyoscyameae clade, where it is placed by Tetenyi (1987). The monotypic *Nicandra* (and tribe Nicandreae) likewise is isolated within the Solanoideae and may represent an early diverging lineage (Figs. 4, 5). *Nicandra* is possibly related to *Exodeconus* (Figs. 4, 5), previously classified in the Solaneae, with which it shares a native distribution in coastal Peru. D'Arcy (1991) suggested that the tribe Solaneae is "inconveniently large," but that appropriate divisions are not readily apparent. The tribe Datureae forms one of several lineages within the Solaneae in the cpDNA tree, suggesting that the Solaneae should be split and that tribes or subtribes may be circumscribed along phylogenetic lines. A more extensive survey of the Solanoideae, currently in progress, should help clarify tribal boundaries and relationships in the subfamily.

Attempts were made using two approaches, each with different underlying assumptions, to gain greater resolution within the Solanoideae. In the functional outgroup approach (Watrous & Wheeler, 1981), the more distantly related taxa in the study are removed and only the closest sister group to the Solanoideae is retained and used as the outgroup. This approach risks sacrificing global parsimony (Maddison et al., 1984) in order to gain resolution by eliminating a large amount of spurious restriction site similarity. The weighted parsimony analysis assumes that equal weighting of all restriction site changes, both gains and losses, does not accurately reflect the probability of gains versus losses of restriction sites (Albert et al., 1991). The fact that both the functional outgroup (Fig. 4) and weighted parsimony (Fig. 5) analyses yielded largely congruent estimates of relationships within the Solanoideae suggests that the weakly supported patterns of relationship implied by each individual analysis may reflect actual phylogenetic history. A comparison of the two approaches used here shows that the weighted parsimony method provided greater resolution than the functional outgroup method (i.e., one shortest tree vs. three), but that the functional outgroup method identified greater character support for the critical, closely spaced branch points that were unresolved in the original analysis. The scant character support for branchings at the base of the Solanoideae is reflected in the very low bootstrap values associated with them (Fig. 4) and suggests a relatively rapid diversification within the subfamily.

Asymmetrically weighted parsimony has been proposed (Albert et al., 1991) as a method for analyzing restriction site data that is preferable to Wagner parsimony (for the above-stated reason) and Dollo parsimony. Dollo parsimony, which allows only a single gain of a character state and prefers the tree with the fewest losses, has been suggested by Debry & Slade (1985) to be more appropriate than Wagner parsimony for use with restriction site data. However, the absolute restriction against parallel gain of a restriction site imposed by Dollo parsimony is viewed as too restrictive (Albert et al., 1991). Two justifications might be advanced for the weighted parsimony approach: (1) that the assumptions concerning restriction site change are more realistic and (2) that greater resolution can be achieved than is often possible using Wagner parsimony. Any phylogenetic analysis is an approximation of the true phylogeny and can be only as reliable as the assumptions underlying the method; therefore, the first point seems to be sufficient justification for asymmetric weighting. The second point is not valid justification in our view, because the resolution (i.e., number of shortest trees) achieved is a function of the asymmetry in weight assigned. A justification based simply on achieving greater resolution should be viewed with caution, bearing in mind that, as an approximation, a cladogram may carry implications concerning relationships that vary in strength as the distribution in character support varies. For example, with this data set, an asymmetric weighting of 1.9 : 1.0 yields three shortest trees, whereas a weighting of 2.0 : 1.0 yields 13 trees (results not shown). The consensus trees from both analyses are congruent and it is doubtful that the greater resolution of the former offers a significantly better approximation

of the phylogeny. Instead of relying only on a weighted parsimony analysis to discriminate among many nearly equal trees, alternate methods for assessing the strength of relationships should be used, including testing alternative topologies, bootstrap analysis, successive analyses using functional outgroups, and decay analysis (to determine how many steps longer the best tree is in which a given clade of interest fails to hold up). With very large data sets the computer time required for some analyses (e.g., weighted parsimony, bootstrap, decay analysis) may be excessive, and more approximate methods may be required. Nevertheless, adequate taxonomic sampling should be a primary consideration for parsimony analyses of large and divergent groups, because a more approximate analysis with a well-represented taxonomic sampling may yield more accurate results than an exact analysis of an inadequately sampled study group (Swofford & Olsen, 1990; Olmstead et al., 1992).

NOLANOIDEAE

The cpDNA tree clearly places *Nolana* within the Solanoideae in a clade with the Lycieae. The association of *Nolana* with *Lycium* has been made by Carlquist (1987) on the basis of wood anatomy, and by Armstrong (1986) on the basis of calyx vasculature. If the subfamily Nolanoideae is recognized, then the Solanoideae would not be strictly monophyletic. Carpel morphology—the primary basis for the maintenance of the distinct family Nolanaceae—has long been a heavily weighted character in traditional angiosperm classifications. Even recent treatments (Thorne, 1968; D'Arcy, 1979), which include *Nolana* and *Alona* in the Solanaceae, maintain a sharp taxonomic distinction through the creation of a new subfamily, Nolanoideae, while admitting that "most of its morphology corresponds to that of the Solanoideae" (D'Arcy, 1991). We suggest that it is time to deemphasize gynoecial morphology for defining higher taxonomic classes and, instead, define taxonomic classes on hypotheses of phylogenetic relationship whenever such schemes are available. In the case of *Nolana*, it seems entirely justified to place it in the tribe Nolaneae in the Solanoideae or, perhaps even more appropriately, in the tribe Lycieae, to which it is closely related molecularly (Figs. 2–5) and anatomically (see above).

CHARACTER EVOLUTION

Characters that distinguish subfamilies in the Solanaceae can be polarized by reference to the hypothesis of phylogeny derived from cpDNA. Defining characters of the Solanoideae, including discoidal seeds containing curved embryos, small pollen grains, and berrylike fruits, should all be viewed as derived traits within the family, whereas the respective states of these characters in the Cestroideae are primitive. D'Arcy (1979, 1991), following the criteria of Melchior (1964), pointed out that the Cestroideae exhibit more advanced characteristics than the Solanaoideae. Likewise, Armstrong (1986) considered cestroid floral anatomy to be advanced and solanoid floral anatomy to be primitive on the basis of a priori assumptions of trends in angiosperm evolution. In the Solanaceae, criteria of advancement not derived from a phylogenetic analysis of the family prove to be in error. It has been recognized that the Solanoideae are more homogeneous in chromosome number and many other attributes than the Cestroideae and that the latter is "somewhat discordant as a taxonomic unit" (D'Arcy, 1991). In light of the cpDNA tree, both observations may be taken as evidence that members of the Solanoideae share a more recent common ancestry than do members of the Cestroideae.

Chromosome base number has been cited commonly (D'Arcy, 1979), along with the morphological characters discussed above, as a trait distinguishing subfamilies. The Solanoideae are almost uniformly $x = 12$, whereas in the Cestroideae base number is more variable, with most genera having base numbers lower than 12. Raven (1975) postulated $x = 7$ for the subclass Asteridae and $x = 7$ for the Convolvulaceae, but $x = 12$ for the Solanaceae, with aneuploid reduction to $x = 7$, as in *Petunia*. The prominent exceptions in the Cestroideae are *Nicotiana*, with $x = 12$ (Goodspeed, 1954), and the Anthocercideae, with $n = 30$ or 36 (Haegi, 1986), which is probably based on $x = 12$. In light of the phylogenetic relationships suggested by the cpDNA analysis, the presence of $x = 12$ should not be considered as ancestral in the Cestroideae, nor be interpreted as a parallelism with the Solanoideae. Rather, $x = 12$ represents a synapomorphy (Fig. 6) uniting the Solanoideae with the Anthocercideae, and part of the Nicotianeae (*Nicotiana* and, perhaps, *Combera*, *Pantacantha*, and *Benthamiella*, but excluding *Petunia*, *Latua*, *Fabiana*, and *Nierembergia*).

Floral zygomorphy, exhibited to a varying extent in the asymmetry of the corolla, but more discretely in the reduction in anther number, has long been used as the defining characteristic of the tribe Salpiglossideae (Wettstein, 1895; Hunziker, 1979). Other treatments have placed the Salpiglossideae in the Scrophulariaceae (Bentham, 1876) or in a

family of its own (Hutchinson, 1969). However, D'Arcy (1978) concluded that the Salpiglossideae are an artificial assemblage of independent lineages in which stamen reduction and corolla asymmetry have arisen. His reasoning, which was not clearly stated in his original paper (D'Arcy, 1978), follows from the floral morphological evidence, which indicates that different pathways are responsible for the development of floral asymmetry in various genera of Salpiglossidae, hence that the plants were probably not closely related (D'Arcy, pers. comm.). The phylogenetic hypothesis presented here supports D'Arcy's conclusion and suggests that floral zygomorphy has evolved independently in at least three lineages within the Cestroideae (Fig. 6).

BIOGEOGRAPHY

Two alternative processes have been postulated to account for the extant distribution of the Solanaceae (D'Arcy, 1991): Present distributions represent (1) vicariant remnants of a former Gondwana distribution (Hawkes & Smith, 1965), or (2) numerous long-distance dispersal events. These two alternatives carry different implications. For the vicariance argument to be correct, the ancestral Solanaceae must have been in the right place (i.e., Southern Hemisphere) at the right time (i.e., prior to the break-up of Gondwana), but dispersal ability is relatively unimportant due to the continuous land connection. For the long-distance dispersal hypothesis to be correct, time of origin is less important, but high dispersability is essential.

The cpDNA tree (Fig. 2) does not indicate absolute dates of origin or diversification, but it does imply relative timing of divergence among lineages by the order of branching on the tree. The predominantly South American Cestroideae were clearly in the right place and were well diversified prior to the appearance of the Solanoideae. If the vicariance hypothesis is viable for the family as a whole, an appropriate distribution should be exhibited by the Cestroideae. However, only two cestroid groups, the Anthocercideae and *Nicotiana*, exhibit disjunctions consistent with a vicariance explanation. Both occur entirely or in part within Australia and have their closest extant relatives in South America. One of these, *Nicotiana*, can be ruled out as a recent event (Martin & Dowd, 1984; Olmstead et al., 1990), leaving a single possibility of a vicariant distribution in the Cestroideae, the Anthocercidae. The Solanoideae are of much more recent origin, but contain many more lineages with worldwide distributions (D'Arcy, 1991). If the global distribution of the Solanoideae is the product of the break-up of Gondwana, then one might expect the more ancient Cestroideae to exhibit a similar pattern.

Seed dispersibility, on the other hand, exhibits an association that is consistent with a long-distance dispersal hypothesis to account for much of the global distribution of the Solanaceae. Animal dispersal of fleshy fruits is commonly associated with long-distance dispersal and the colonization of oceanic islands (Carlquist, 1974), whereas dry capsular fruits and small, unornamented seeds tend to be local in their dispersal. The animal-dispersed, fleshy-fruited Solanoideae have many lineages that exhibit intercontinental distribution. Most notably, *Solanum* subg. *Leptostemonum*, which is one of the most widely distributed groups in the family, is implied by the cpDNA tree to be of recent origin (*S. candidum* and *S. carolinense* in Fig. 2) relative to most of the Solanaceae.

D'Arcy (1991) concluded that the pattern of geographic distribution in the family is likely to be the product of both long-distance dispersal and vicariance resulting from continental drift, their relative importance depending upon the age of the family. Our cpDNA analysis argues against vicariance and in favor of long-distance dispersal to account for the distribution of most solanaceous taxa with intercontinental distributions. The best case for a vicariant distribution may be the Anthocercideae, but even here only a single lineage in an already substantially diversified Cestroideae is found outside the New World (except for the recent colonization of Australia and Africa by *Nicotiana*). This suggests that the origin, or at least much of the early diversification of the Solanaceae (i.e., Cestroideae), appears to have followed the split-up of Gondwana and the disappearance of land connections among the continents of the Southern Hemisphere approximately 50 million years ago (Parrish, 1987).

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EVIDENCE FOR A POLYPHYLETIC ORIGIN OF THE LABIATAE[1]

Philip D. Cantino[2]

ABSTRACT

A preliminary cladistic analysis suggests that the Labiatae are polyphyletic as presently circumscribed. The gynobasic-styled Labiatae emerge as a clade, nested within a larger group characterized by suprareticulate pollen and a fruit composed of nutlets. The latter includes the bulk of the Labiatae plus the verbenaceous genera *Garrettia* and *Holmskioldia*; its closest relatives are in tribe Viticeae (Verbenaceae). In contrast, *Teucrium* and five other genera of Ajugeae (Labiatae) belong to a large clade characterized by pollen with branched to granular columellae, most members of which are currently assigned to tribes Clerodendreae and Caryopterideae (Verbenaceae). Another group traditionally placed in the Labiatae, tribe Prostanthereae, appears to be most closely related to subfamily Chloanthoideae (Verbenaceae). The hypothesis that the gynobasic-styled Labiatae evolved in southern China or Indomalaysia (Wu & Li, 1982) is supported by this analysis. An Australian origin is hypothesized here for the cosmopolitan genus *Teucrium* based on the distributions of its closest relatives.

The Labiatae, one of the largest and most distinctive angiosperm families, have long been considered a "natural" group (in the pre-Darwinian sense of the word; see Stevens, 1984). It is often tacitly assumed that such a group is monophyletic. However, palynological evidence (Abu-Asab, 1990; Abu-Asab & Cantino, 1992) suggests that the Labiatae are polyphyletic as traditionally circumscribed. This hypothesis is tested here by means of a cladistic analysis of mainly morphological and anatomical data.

THE POLYPHYLY HYPOTHESIS

CURRENT CLASSIFICATION OF THE LABIATAE

The classification of the Labiatae that is most widely used today (Briquet, 1895–1897) is based heavily on the work of Bentham (1832–1836, 1848, 1876). Briquet subdivided Bentham's taxa more finely, increased the rank of some of them, and reclassified a few genera, but his treatment differs from Bentham's in only one fundamental way: Briquet recognized a large subfamily Lamioideae ("Stachyoideae"), which is at best paraphyletic and probably polyphyletic (Cantino & Sanders, 1986). Neither Bentham nor Briquet attempted to reconstruct the phylogeny of the family, and no phylogeny of the Labiatae has yet been published.

An alternative classification of the Labiatae was proposed by Erdtman (1945) on the basis of palynological features. He divided the family into two subfamilies: Lamioideae, with tricolpate pollen shed in a two-celled stage, and Nepetoideae, with hexacolpate pollen shed in a three-celled stage. This division correlates well with a variety of embryological and phytochemical characters (Wunderlich, 1967; Zoz & Litvinenko, 1979; Cantino & Sanders, 1986). Erdtman's subfamilial classification is highly congruent with Bentham's (1876) tribal classification, four of Bentham's tribes composing subfamily Lamioideae, and the other four composing subfamily Nepetoideae (Cantino & Sanders, 1986).

A numerical phenetic study conducted by El-Gazzar & Watson (1970) provided further support for Erdtman's subfamilies. Their results cast doubt on the phenetic cohesiveness of some of Bentham's and Briquet's groupings, but the two principal branches of their phenogram correspond to Erdtman's subfamilies. Although Erdtman's subfamilies appear to be primary phenetic units of the Labiatae,

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[2] Department of Botany, Ohio University, Athens, Ohio 45701, U.S.A.

TABLE 1. Taxa included in study group. Numbers in parentheses: number of genera included in this analysis/ number of genera in the taxon. Parenthetical acronyms are used in Table 2 and Figures 1–4.

Verbenaceae sensu lato
Caryopteridoideae (6/6)
Caryopterideae (5/5) (CAR)
Teijsmanniodendreae (1/1) (TEI)
Chloanthoideae (10/10)
Chloantheae (5/5) (CH)
Physopsideae (5/5) (PH)
Verbenoideae (2/ca. 38)
Monochileae (2/2) (MO)
Viticoideae (26/28)
Callicarpeae (3/4) (CAL)
Clerodendreae (10/10) (CL)
Tectoneae (3/3) (TEC)
Viticeae (10/11) (VI)
Labiatae
Lamioideae (32/ca. 83)
Ajugeae (12/12) (AJ)
Prostanthereae (7/7) (PR)
Scutellarieae (4/4) (SC)
Gynobasic-styled Lamioideae (8/ca. 60) (GL)
Nepetoideae (4/ca. 160) (NE)

the question remains whether they are monophyletic. Synapomorphies can be demonstrated for subfamily Nepetoideae but not for subfamily Lamioideae (Cantino & Sanders, 1986). The latter group is of interest because it includes the two tribes (Ajugeae and Prostanthereae) that are intermediate in gynoecial morphology between the other Labiatae and the Verbenaceae.

POLYPHYLY OF LABIATAE: INITIAL EVIDENCE

It is widely accepted that the Labiatae evolved from the Verbenaceae, the latter thus being paraphyletic. The two families form the core of the order Lamiales of Cronquist (1981), Dahlgren (1983), Takhtajan (1987), and Thorne (1983). They have traditionally been distinguished on the basis of stylar position—terminal in the Verbenaceae and gynobasic in the Labiatae. However, in two tribes of Labiatae the gynoecium is intermediate in structure; the ovary is only shallowly four-lobed and the style is sunken but not fully gynobasic. Within tribes Ajugeae and Prostanthereae, the ovary may be essentially unlobed (e.g., *Amethystea*, *Schnabelia*), lobed a third to halfway to the base (many members of both tribes), or, rarely, lobed as much as three-quarters of the way to the base with the style thus almost gynobasic (e.g., *Microcorys longifolia* Benth.). Indeed, this full range of gynoecial structure occurs within *Microcorys* (Prostanthereae). In the Verbenaceae, the ovary is usually unlobed but may be lobed as much as halfway to the base (e.g., some species of *Oxera*). In summary, there is a continuum in the degree of ovary lobing, with some intermediates assigned to the Labiatae and some to the Verbenaceae. Since this is the only character distinguishing the two families as currently circumscribed, the taxonomic limits of the Labiatae are unclear, and there is no synapomorphy supporting their monophyly.

On the contrary, a palynological survey of subfamily Lamioideae has provided evidence that tribe Ajugeae (and hence the Labiatae as well) is polyphyletic, its component genera having arisen independently from several different lineages of Verbenaceae (Abu-Asab, 1990; Abu-Asab & Cantino, 1992). Derived pollen features appear to delimit three clades that transcend the family boundary, comprising the following taxa (hypothesized synapomorphies in parentheses): (1) the gynobasic-styled Labiatae, tribe Scutellarieae, six genera of Ajugeae and at least two genera of Verbenaceae (suprareticulate sculpturing); (2) *Teucrium* (Ajugeae) and three genera of Verbenaceae (verrucate sculpturing, operculate colpi); (3) five genera of Ajugeae and ten genera of Verbenaceae (spinulose sculpturing). The latter two clades are linked to each other and to a few other genera of Verbenaceae with different forms of sculpturing by their shared possession of branched columellae (varying to granular in a few taxa), a hypothesized synapomorphy.

MATERIALS AND METHODS

THE STUDY GROUP

Because the question of primary interest concerns the origin of the Labiatae, the study group (Table 1) centers on the primitive Labiatae (viz., tribes Ajugeae and Prostanthereae) and those groups of Verbenaceae sensu lato that appear to be most closely related to the Labiatae (viz., subfamilies Caryopteridoideae, Chloanthoideae, and Viticoideae). Of the genera that compose these groups, only *Adelosa* Blume and *Archboldia* E. Beer & H. J. Lam (Viticoideae) have been omitted from the analysis because of unavailability of material for study.

These three groups of Verbenaceae share with the Labiatae a distinctive and possibly synapomorphic ovary structure, in which the carpel walls recurve into the interior of the carpel, with the ovules borne short of the carpel margins (Junell, 1934). The group thus delimited may also include the segregate families Avicenniaceae and Sympho-